THE SUPEROXIDE GENERATING OXIDOREDUCTASE OF HUMAN NEUTROPHILS: METAL ION DEPENDENCE AND DELINEATION OF ITS CATALYTIC COMPONENTS. Terrence R. Green, Dept. Clinical Pathology, Veterans Adm. Med. Ctr. and Oregon Health Sciences University, Portland, OR.

The NADPH dependent superoxide generating oxidoreductase of human neutrophils requires calcium and magnesium for full expression of its catalytic activity. In addition, it exhibits NADPH dependent DCIP reductase and cytochrome c reductase activities, and duroquinol dehydrogenase activity. NADPH and duroquinol induce formation of a 450 nm difference spectrum. The latter substrate-induced spectra are similar to that of myeloperoxidase compound-III. None of the NADPH dependent components are expressed in enzyme fractions from resting cells, nor in fractions from stimulated cells of a patient with chronic granulomatous disease. These results demonstrate that the oxidoreductase is a multienzyme complex of linked redox reactions. A catalytic model of the complex will be presented based upon these observations and dithionite difference spectra demonstrating the presence of a cytochrome b type chromophore in association with the oxidoreductase.

The microbicidal oxygen radical-producing oxidase of human neutrophils

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Neutrophil plasma membranes contain both cytochrome b of low midpoint potential ($E_{m7.0}$ = -245mV) and FAD (ratio approx. 1:1). The cytochrome reacts fast with oxygen and binds CO : these are properties expected of an oxidase. In male patients (19 cases) with a defective oxidase (chronic granulomatous disease) the cytochrome is absent and FAD much diminished. In carriers of this X-linked disease (24 cases) the cytochrome is present at subnormal concentration. Cultured undifferentiated bone marrow cells (HL 60) lacked microbicidal oxidase and cytochrome b_{-245} : induction of the oxidase is accompanied by induction of cytochrome b_{-245}.

MEASUREMENT OF HYDROPEROXIDES AND COLLAGEN ELASTICITY DIRECTLY IN VIVO IN MICE AND MAN. R.D. Lippman, Med. Cell Biol., Univ. Uppsala, Uppsala, Sweden S-75123.

Hydroperoxides and amide bonding in mice and man were measured directly in vivo using near infrared spectroscopy. Middle-aged mice decreased 10-fold hydroperoxide levels (HPL) when fed strong antioxidants. However, diets supplemented with high doses of vitamin E and related derivatives yielded HPL higher than controls. Hydroperoxide levels in middle-aged rabbits and humans were ~3 and ~8 times those of middle-aged mice controls. Hydroperoxide levels remained constant during the growth phase of mouse life while increasing 20-fold during the senescent phase. Significant increases in amide bonding and consequent collagen inelasticity were determined in "teenage" and"retirement age" mice. Abstinence from antioxidant-supplemented diets resulted in large increases of amide bonding and HPL. However, butylated hydroxytoluene (BHT) and related strong antioxidants gave lasting prophylatic effects.

In human clinical studies, strong antioxidants such as ethoxquin, BHT and ACF lowered HPL 15% versus placebo during 1.5 to 4 h periods. Single dosages of 300 mg or less vitamin C lowered HPL 6% while dosages in excess of 1 gm raised HPL 3-10% versus placebo. A theraputic or "prophylatic window" of optimal antioxidant/prooxidant dosages in man was established using this pharmacokinetic method.

Mice fed strong antioxidant supplements during a 4 month period only showed increases in both mean and maximun lifespan versus controls. This finding suggests that aging mice fed strong antioxidant supplements (10 to 25% of the LD_{50}) during their "retirement age" are unable to attain increases in maximum lifespan due to increasing antioxidant toxicity. That is to say, a decreasing physiologic ability to detoxify high doses of strong antioxidants during senescence outweighs any life extending benefits.